REMARKS

Reconsideration of this application in view of the following remarks is respectfully requested.

I. Status of the claims

Claims 1-5, 10-11, 17 and 25-36 are pending in this case. Claims 1, 2 and 17 are amended to further clarify the scope of the invention. New claims 29-36 are added. Support for amendment and new claims can be found, for example, on page 1, last paragraph; page 3, second paragraph; page 4, lines 7-11 and 14-15; page 5, last paragraph; and page 14, Example 1. Thus, no new matter has been introduced as a result of the amendment.

II. Rejection under 35 U.S.C. § 112, second paragraph

Claims 2-5, and 10-11 are rejected under 35 USC 112, second paragraph as being indefinite. Specifically, the Examiner asserted that it is not clear what "equivalent to about 50 mM to about 150 mM" means. Applicants have amended claim 2 to recite ionic strength "equivalent to from about 50 mM to about 150 mM salt equivalent." Applicants respectfully submit that the amendment has rendered the rejection moot.

III. Rejections under 35 U.S.C. § 103(a)

A. Rejections under 35 U.S.C. § 103(a) based on Newton in view of Sporeno and Gaberc-Porekar

Claims 1-5, 10-11 and 17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Newton et al. (Mol. Biotech. 20:63-76 (2002)) ("Newton") in view of Sporeno et al. (Cytokine, 6(3):255-264 (1994)) ("Sporeno") and Gaberc-Porekar et al. (J. Biochem. Biophys. Methods 49:335-360 (2001)) ("Gaberc-Porekar"). Specifically, the Examiner asserted that Newton teaches a method for purifying a 6x histidine-tagged protein from a protein preparation comprising concentrating the tagged protein with a negatively charged capture support, eluting the tagged protein from the capture support, and purifying the tagged protein from the eluate with a tag-specific affinity support. The Examiner further asserted that Sporeno teaches a method of purifying a 6x histidine tagged cytokine with a four-helix bundle motif, and Gaberc-Porekar teaches that protein purification using engineered histidine tags in combination with the Ni^{2+} matrix often achieves over 90% purity in one step. Thus, the Examiner concludes that it would have been obvious to one skilled artisan to modify the method of Newton by applying thereto the polyhistidine-tagged cytokine with a four-helix bundle motif of Sporeno. Applicants respectfully disagree.

A claimed invention is unpatentable if the differences between it and the prior art "are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art." 35 U.S.C. § 103(a); see *Graham v. John Deere Co.*, 383 U.S. 1, 14 (1966). The ultimate determination of whether an invention is or is not obvious is based on underlying factual inquiries including: (1) the scope and content of the prior art; (2) the level of ordinary skill in the prior art; (3) the differences between the claimed invention and the prior art; and (4) objective evidence of nonobviousness. *See Graham*, 383 U.S. at 17-18.

The Supreme Court emphasizes that the key of supporting any rejection under 35 U.S.C. §103 is the clear articulation of the reason(s) why the claimed invention would have been obvious. *KSR Int'l Co. v. Teleflex Inc.*, No. 04-1350, slip op. at 14 (2007). The Court, quoting *In re Kahn*, stated that "rejections on obviousness cannot be sustained with mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." *In re Kahn*, 441, F.3d 977, 988 (Fed. Cir. 2006). Similarly, Office personnel must explain why the differences between the prior art and the claimed invention would have been obvious to one of ordinary skill in the art. Federal Register Vol.72, No. 195, October 10, 2007. Applicants respectfully submit that the Examiner has not established a *prima facie* case of obviousness based on the arguments as set forth below.

The cited art has different scopes and intends to solve different problems from those of the claimed invention

Applicants respectfully traverse the rejection but have nevertheless amended the claims. Amended claims 1 and 17 are directed to a method for purifying a polyhistidine-tagged cytokine from a protein preparation that is derived from a mammalian cell culture, wherein the polyhistidine-tagged cytokine is present in the protein preparation at a concentration of no more than 2mg/L, said method comprising, *inter alia*: (a) concentrating the polyhistidine-tagged cytokine in the protein preparation with a negatively charged capture support, wherein the negatively charged capture support comprises heparin, and (b) purifying the polyhistidine-tagged cytokine from the eluate of step (a)(iii) with a tag-specific affinity support. Dependent claims further define the invention by reciting: wherein the polyhistidine-tagged cytokine is poly-histidine-tagged human IL9ra (claims 26 and 28); wherein the protein preparation is derived from a mammalian cell culture supernatant (claims 30 and 34), and is nearly 100% captured from the protein preparation with greater than 99% purity (claims 32 and 36); or wherein the polyhistidine-tagged cytokine is transiently expressed in the mammalian cell culture (claims 31 and 35).

Despite the reported success in using the Ni^{2+} matrix to purify polyhistidine-tagged proteins

that were abundantly expressed in bacteria, the same success has not been easily attainable in purifying proteins expressed in low quantities, a problem often seen in mammalian cell expression systems. Applicants described in the specification that at low concentrations, the binding of a polyhistidine-tagged target protein to the Ni^{2+} matrix is often thermodynamically unfavorable. See page 1, last paragraph of the specification. As a result, less than 25% of the target protein may be bound out of culture supernatant to the affinity matrix. *Id.* Applicants discovered that implementing a chromatography-based concentration step before the affinity purification step utilizing a heparin capture support greatly improves the binding thermodynamics of the target protein to the Ni^{2+} affinity matrix. See page 2, last paragraph of the specification. The concentration step is particularly beneficial to purification proteins present in low quantities because an enrichment of the target protein by several hundred fold can be achieved, which makes possible quantitative capture of the target protein by the N^{2+} affinity matrix. See page 2, last paragraph.

In contrast, the nature of the problem to be solved by Newton is completely different. Newton merely relates to a method for purifying polyhistidine-tagged RNase single-chain antibody fusion protein, which is expressed in large quantities in bacteria cells and accumulated in bacterial inclusion bodies. Thus, Newton at best teaches a method for purifying an abundantly expressed recombinant protein from the inclusion bodies in a bacterial expression system. It is in this context that Newton advised against direct application of a protein preparation to the Ni^{2+} column. See page 73, note 44 of Newton. Newton, however, does not provide *any* guidance as to how to purify a recombinant protein expressed in *low quantities* in a *mammalian* expression system. Newton does not teach or suggest that the method disclosed has general applicability to all target proteins, much less to a recombinant protein expressed in minute quantities in a mammalian expression system. In fact, Newton states that the best method for purification needs to be determined for each specific target protein. See page 68, point No. 5 under section 3.8 of Newton.

It would not have been obvious to one skilled artisan to apply the two-step purification scheme of Newton to arrive at the claimed invention because Newton does not recognize nor appreciate the unique problems in purifying recombinant proteins expressed in low quantities in mammalian cells. Newton merely relates to purification of abundantly expressed proteins in bacterial inclusion bodies. Newton does not concern purification of a polyhistidine-tagged cytokine from a mammalian cell culture lysate or supernatant, much less improving the binding thermodynamics of the recombinant protein to the N^{2+} matrix. Newton does not recognize the problem and certainly does not appreciate the solution that an additional concentration step would greatly improve the binding thermodynamics of the dilute cytokine to the N^{2+} matrix. Newton certainly does not teach or suggest a method for purifying a polyhistidine-tagged cytokine expressed in low quantities in

mammalian cells, wherein the polyhistidine-tagged cytokine is nearly 100% captured from the protein preparation with greater than 99% purity.

Sporeno does not cure the defect. Sporeno merely relates to a single-column affinity purification scheme for purifying a polyhistidine-tagged OncM protein in a bacterial expression system. Sporeno teaches over-expressing the target protein in bacteria cells, isolating inclusion bodies, refolding the protein and applying the protein preparation directly onto a Ni^{2+} column. Although Newton suggests that the Ni^{2+} column should not be used as the first column, Sporeno nevertheless successfully demonstrated purification of a polyhistidine-tagged cytokine abundantly expressed in bacteria directly through the Ni^{2+} column. Similar to Newton, Sporeno does not recognize the unique problems faced in purifying polyhistidine-tagged proteins expressed in low quantities in mammalian cells. Sporeno does not provide any guidance regarding how to purify a polyhistidine-tagged cytokine expressed in low quantities in mammalian cells, let alone a purification that allows nearly quantitative recovery with greater than 99% purity.

Equally unhelpful is Gaberc-Porekar, which constitutes a general review of immobilized-metal affinity chromatography. Similar to Newton and Sporeno, Gaberc-Porekar does not recognizes or appreciates the unique problems in purifying polyhistidine-tagged proteins expressed in mammalian cell culture in low quantities nor provide any guidance in solving the unique challenges faced by the instant application.

Secondary consideration of unexpected results further support non-obviousness

In addition, Applicants unexpectedly discovered that the claimed method surprisingly overcame the difficulties in purifying low quantities of human cytokines and provided nearly 100% capture of the polyhistidine-tagged cytokines with greater than 99% final purity as judged by silver stain SDS gels. See, for example, page 14 and 15 of the specification. Such unexpected results are not taught or suggested in any one of the cited art, alone or in combination. Examiner's assertion of obviousness is precisely the result of impermissible hindsight reconstruction that is explicitly prohibited by case law. *KSR International Co., v. Teleflex Inc.*, 127 S. Ct. 1727, 1742 (2007). Combining elements that work together "in an unexpected and fruitful manner" would not have been obvious. *See PharmaStem Therapeutics Inc. v. ViaCell Inc.*, 83 USPQ2d 1289. 1302 (Fed. Cir. 2007).

In view of the unique difficulties faced in purifying polyhistidine-tagged proteins expressed in low quantities in mammalian cells, one of skill in the art would not have found it beneficial to resort to the cited art for guidance with a reasonable expectation of success because the art are completely

silent on the problems as well as the solution thereto. It was the Applicants' disclosure that recognized the problems and provided a solution by concentrating the target protein before affinity purification by the Ni^{2+} matrix. The claimed method unexpectedly increased the recovery and purity of the tagged cytokine by overcoming the hurdle of the unfavorable binding thermodynamics to the Ni^{2+} matrix. Such insights are more than optimization of the ranges routinely exercised by one of skill in the art. Implementing a negatively charged capture support to improve the binding thermodynamics in the following affinity purification requires one of skill in the art to exercise no less than the inventive facilities.

Further, the cited art does not teach every element of the claims. None of the cited art, alone or in combination, teaches or suggests purification of a polyhistidine-tagged protein expressed in *mammalian cells* in *low quantities*, much less nearly quantitative recovery of such polyhistidine-tagged protein with over 99% purity. In view of the unique problems faced in purifying proteins expressed in low quantities in mammalian cells, one of skill in the art would not have found obvious the differences between the teachings of the art and the claimed invention. Thus, it would not have been obvious to one skilled artisan to combine Newton with Sporeno and Gaberc-Porekar with a reasonable expectation of success in arriving at the claimed invention.

Based on the foregoing, reconsideration and withdrawal of the rejections under 35 USC §103 are respectfully requested.

B. Rejections under 35 U.S.C. § 103(a) based on Newton in view of Sporeno, Gaberc-Porekar, Lovenberg, and Soussi-Gounni

Claims 1, 17 and 25-28 are rejected under 35 U.S.C. 103(a) as being unpatentable over Newton in view of Sporeno, Gaberc-Porekar, Lovenberg et al. (US. 6,239,268) ("Lovenberg"), and Soussi-Gounni et al. (Molecular Mechanisms in Allergy and Clinical Immunology, April 2001, pp575-582) (Soussi-Gounni). Specifically, the Examiner asserted that Lovenberg teaches purification of a human cytokine using a Ni^{2+} column, and Soussi-Gounni teaches that IL9 may play a role in asthma pathology. The Examiner thus asserted that it would have been obvious to one skilled in the art to purify a human cytokine such as IL9ra by using negatively charged capture support in combination with a Ni^{2+} column.

Applicants respectfully traverse the rejection. As stated above, one of skill in the art would not have found it obvious, based on Newton, Sporeno and Gaberc-Porekar, to apply the claimed two-step purification scheme to purify a polyhistidine-tagged cytokine expressed in low quantities in mammalian cells with a reasonable expectation of success. Neither Lovenberg nor Soussi-Gounni

cures the defects. Contrary to Examiner's assertion, Lovenberg merely mentions in passing the purification of a cytokine *receptor*, NOT a *cytokine*. Col. 5, lines 27-49. Soussi-Gounni merely studies the activity of IL9, and does not teach of suggest purification thereof. In view of the unique difficulties in purifying polyhistidine-tagged protein expressed in mammalian cells in low quantities, one of skill in the art would not have used the two-column purification scheme similar to that of Newton's to purify IL9ra expressed in mammalian cells in low quantities with a reasonable expectation of success. Thus, claims 1, 17 and 25-28 are not obvious based on Newton in view of Sporeno, Gaberc-Porekar, Lovenberg and Soussi-Gounni. Reconsideration and withdrawal of the rejections are earnestly requested.

IV. Conclusion

Reconsideration of this application is respectfully requested and a favorable determination is earnestly solicited. The Examiner is invited to contact the undersigned representative if the Examiner believes this would be helpful in expediting the allowance of this application.

Dated: April 28, 2008

Respectfully submitted,

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